

Revised Protocol for Preparing Leaf Samples Collected in the Field

- 1.** You need a Tupperware container containing a 5% bleach solution. Obtain the number of Petri dishes needed (one per egg mass). Use the larger petri dishes, they vary a little in size but are between 8 and 9 cm in size. Make sure the lids are not too loose or too tight. Use only the Petri dishes that are clean.
- 2.** Next obtain as many large cotton cosmetic pads (about 7.5cm width) that you need. Place the pad in the bottom of the petri dish and use a Nalgene wash bottle to sufficiently wet the cotton pad. Make sure the filter papers are not too wet or dripping. Do not use paper towels.
- 3.** Label the Petri dishes with the site code, leaf code and vial code.
- 4.** If necessary, cut the leaves to fit the Petri dish, making sure that you do not have more than one egg mass per Petri dish.
- 5.** Using a cotton ball, gently clean the leaves in the 5% bleach solution, removing all dirt.
- 6.** Accurately count and record the number of eggs per mass on the data sheets. Note any exit holes, obviously emerged eggs, or damaged eggs. Do not worry about recording if the egg looks dead, this will be analyzed at the end.
- 7.** Place the leaf in the correct Petri dish.
- 8.** Place a rubber band securely around the dish to ensure that the lid stays on and no insects can sneak out of the dish.
- 9.** Place Petri dishes in a Tupperware container labeled with the site code, date of monitoring, and county. You do not need to put the lid on the Tupperware container. Use only Tupperware containers that are clean.